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Porous Composites for Adhering Artificial Cartilage to Bone

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ABSTRACT

Artificial cartilage can be grown from cultured chondrocytes, but adhering this tissue to bone presents a challenge. Porous polymer/bioactive glass composites are candidate materials for engineering the artificial cartilage/bone interface and possibly other soft-to-hard tissue (ligament/bone, tendon/bone) interfaces. A phase separation technique was used to make porous polymer/bioactive glass composites. The composites (thickness: 200-500 μm) have asymmetric structures with dense top layers and porous structures beneath. The porous structures consist of large pores (>100 μm) in a network of smaller (<10 μm) interconnected pores. The dense layers were removed and large pores exposed by abrasion or salt leaching from the casting surface. The tissue bonding abilities of the composites were studied *in vitro* in simulated body fluid (SBF) and in rabbit chondrocyte culture. Culture studies revealed that composite surfaces were suitable for attachment, spreading and proliferation of chondrocytes. The growth of hydroxycarbonate apatite (HCA) inside and on the composites after soaking in the SBF for two weeks demonstrates their potential for integration with bone. The results indicate the potential for the composites to facilitate growth and attachment of artificial cartilage to bone.

INTRODUCTION

Artificial cartilage prepared from cultured chondrocytes offers promise as a treatment of cartilage defects [1], but connecting this artificial soft tissue to bone is difficult. The natural interface between cartilage and bone contains a zone of calcified cartilage [2]. Replicating this calcified interface may be vital to adhering an artificial cartilage to bone. One strategy is to develop a substrate that supports the growth and attachment of cartilage and encourages a calcified zone. In addition, this substrate should also bond to bone on implantation in order to create an engineered interface between artificial cartilage and native bone.

Porous polymer/bioactive glass composites are candidate materials for the artificial cartilage/bone interface and possibly other soft/hard tissue (ligament/bone, tendon/bone) interfaces. A porous polymer matrix with large (>100 μm) pores and small (5-10 μm) interconnected pores would provide a means of biological bonding by cell attachment and ingrowth. The polymer matrix may also provide flexibility and toughness. Bioactive glass bonds well to both hard and soft tissues [3], so the incorporation of bioactive glass particles in the composite will enhance bonding ability. It is also possible to control mineralization in the composite by changing the glass content because the presence of bioactive glass particles enhances the composites' apatite formation ability. Ceramic particles incorporated in the polymer matrix may also strengthen and stabilize the porous polymer matrix. This paper

describes the preparation and morphologies of porous polymer (polysulfone, polyurethane and polylactide)/bioactive glass composites, and gives the two-week results for *in vitro* apatite formation in the simulated body fluid (SBF) and *in vitro* compatibility with rabbit chondrocytes.

EXPERIMENTAL PROCEDURE

Polysulfone powders (M_w = 35,000), tetrahydrofuran (THF), N, N-dimethylacetamide (DMAc), N,N-Dimethyl Formamide (DMF), 1,4-dioxane and ethanol were obtained from Aldrich Chemical Company. Polyurethane used in this research was industrial grade Avalon 92AE (from Huntsman Polyurethanes Co.). Polylactide (M_w ~ 80,000) was synthesized as previously reported [4]. Bioactive glass particles with an average particle size of about 10 μm and a composition of 4.6MgO, 44.7CaO, 34.0SiO_2, 16.2P_2O_5 and 0.5CaF_2 (wt%) was purchased from Specialty Glass, Inc. Some of the bioactive glass was further ground in an attrition mill to achieve an average particle size of approximately 2.0 μm.

Porous polymer/bioactive glass composites prepared by the phase separation technique, which was originally designed for porous polymeric membranes [5], were made as reported before [6]. Briefly, homogeneous composite dispersions with different compositions were made by combining polymer, bioactive glass particles, solvents (THF and DMAc for polysulfone, DMF for polyurethane and dioxane for polylactide) and non-solvent (ethanol for polysulfone, water for polyurethane and polylactide). The dispersions were cast onto glass substrates by a doctor blade (gap height = 900 μm). The resultant coatings were either dried in air for about 10 seconds and then immersed in a water bath (for polysulfone composites) or immediately immersed in a water bath (for polyurethane and polylactide composites) for at least 10 minutes to induce phase separation. A solvent exchange was also performed for polysulfone composites in a methanol solution. The composites were dried at room temperature for at least 24 hours before further drying in a vacuum oven. Scanning Electron Microscopy (SEM, Hitachi S800 and S900) was used to characterize the microstructure of the porous polymer/bioactive glass composites.

Some porous polysulfone/bioactive glass composites were abraded by 400-grit SiC paper to remove top and bottom surfaces. Bottom surface pores can also be developed by casting composite dispersions on a layer of NaCl particles (size < 212 μm by using USA Standard Testing No. 70 Sieve) that were held in place on the glass substrate via double-sided tape. The NaCl particles were leached away from the composites during the phase separation in the water bath.

Surface-abraded porous polysulfone/ bioactive glass (9.4 vol%) composites were sterilized using ethylene oxide. Rabbit chondrocytes were isolated as previously reported [1]. Cells were cultured on the sterilized porous polysulfone/ bioactive glass composites in 12-well plates (approximately 22 mm in diameter). Type I collagen (Vitrogen® collagen) was chosen as a control. Dulbecco's Modified Eagle Medium (DMEM) with 15% fetal calf serum (Life Technologies, Rockville, MD) and other additives as in [2] were used as culture medium. Cells were fed three times per week by replacing the old medium. After 2 weeks of culturing, the samples were washed with saline and analyzed. Cell density (cells/mm^2) was calculated with the aid of an optical microscope with a calibrated reticle. Samples were stained for alkaline phosphatase using the Genius alkaline phosphatase detection system from Boehringer Mannheim with a Nuclear Fast Red counterstain. The percent of cells that expressed alkaline phosphatase activity was then calculated. Alkaline phosphatase activity serves as a measure of the ability to induce hypertrophy and mineralization.

An *in vitro* apatite growth test was carried out by soaking the composites (sample size~1 cm^2) in 50 mL SBF at 37 °C. The composition and preparation of SBF were described by Kokubo et al. [7]. SBF was changed every other day and after 2 weeks of soaking, composites were characterized by SEM, X-ray diffraction (XRD) and Fourier transform infrared (FTIR).

RESULTS AND DISCUSSION

Cross-sectional microstructures of the porous polymer/bioactive glass composites are shown in Figure 1. Four features are apparent: large pores with a size more than 100 μm, interconnected small pores with a size of approximately 5 μm, a homogeneous distribution of glass particles and a denser skin layer on the surface that contacted the water bath. The porous structure of the

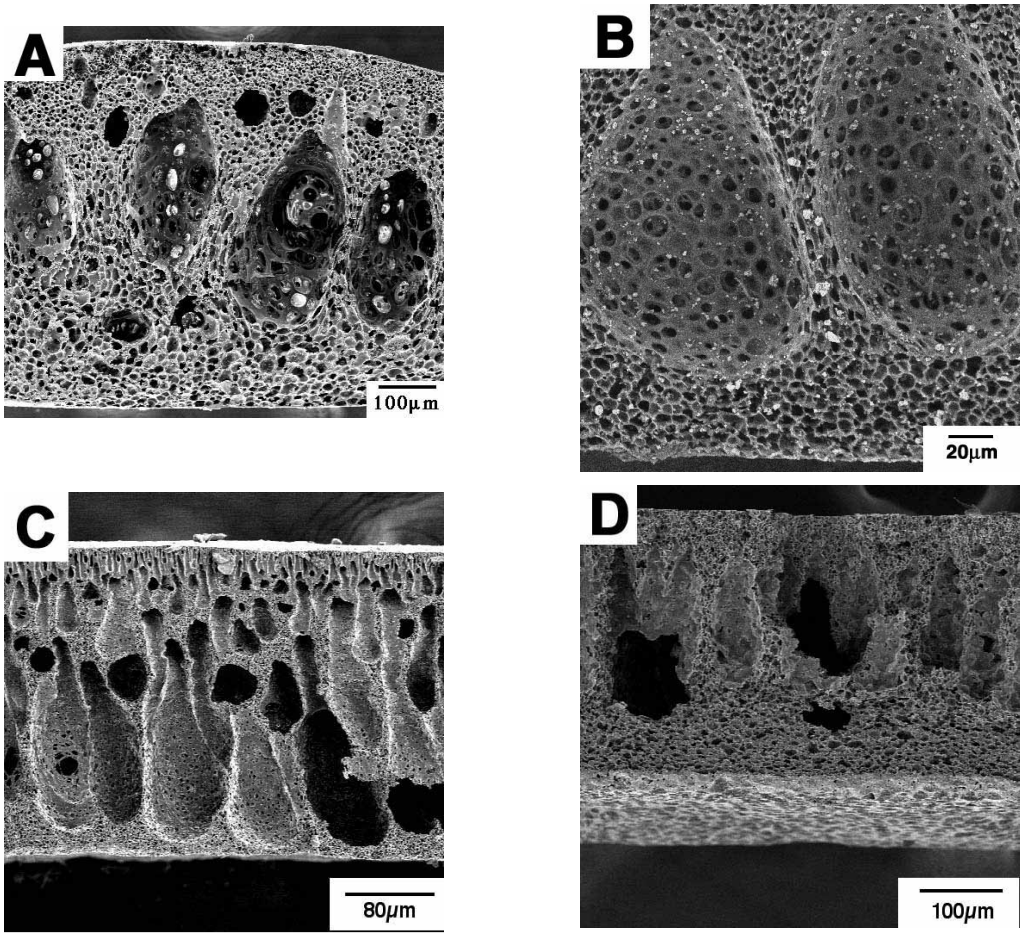

Figure 1. Cross-sectional SEM images of porous polymer/bioactive glass composites with 9 vol% glass. (A) polysulfone; (B) back-scattered image of polysulfone composite ; (C) polyurethane and (D) polylactide.

polymer/bioactive glass composites results from polymer phase separation, in which a homogeneous polymer solution is separated into polymer-rich and polymer-lean phases by changing the polymer solubility through solvent composition. The final microstructure contains a

continuous rigid structure resulting from the polymer-rich phase and pores from the drying of the polymer-lean phase [8].

The dense layers of the composites presented a challenge for the application as porous interface materials. They were removed and large pores (size between 20 and 150 μm) exposed by abrasion or salt leaching from the casting surface (Figure 2). Previous studies also showed that microstructural differences were observed for different polymer molecular weights and concentrations, as well as glass contents and particle sizes (not shown) depending on the choice of the polymer, glass particle content and size, and the interaction between polymer and glass particles [6].

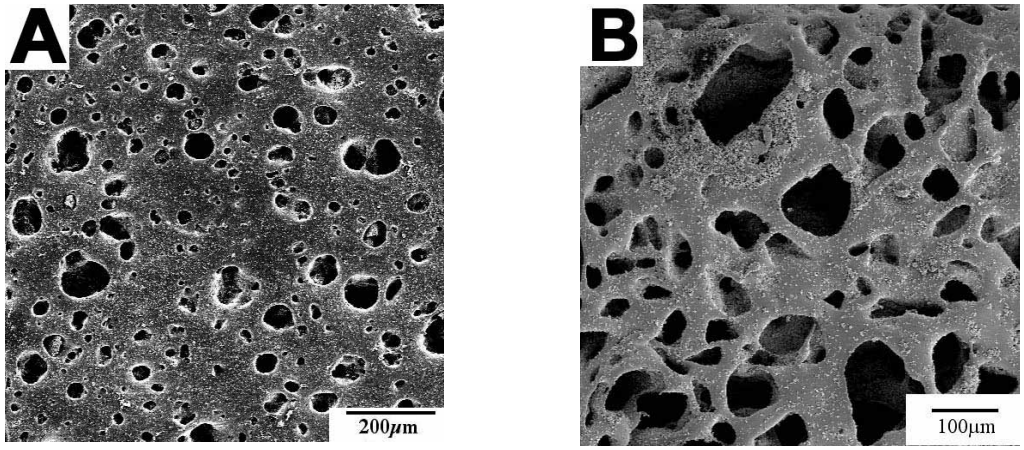

Figure 2. SEM images of the porous polysulfone/bioactive glass (9 vol%) composites' surface after abrasion (A) or salt leaching from the casting surface (B).

Figure 3 shows the chondrocyte cell density and the percent of alkaline phosphatase-positive cells, after culturing rabbit chondrocytes on the porous polysulfone alone, porous polysulfone/bioactive glass composites with 9 vol% glass, and control (Type I collagen). The dense surfaces of these substrates were removed by abrasion prior to culturing the cells. Cells on porous polysulfone /bioactive glass composites had comparable cell density and alkaline phosphatase activity to that found on the control surface. These results demonstrate the suitability of the composites for attachment, spreading, and proliferation of chondrocytes, and are an improvement compared with previous research on porous bioactive glass alone [9]. For the porous bioactive glass, the chondrocyte cell density fell off substantially after 2 weeks in culture. One indicator of a chondrocyte's tendency to take on a hypertrophic phenotype and calcify its matrix is alkaline phosphase activity. Results in Figure 3 show no difference in alkaline phosphatase activity between the composites and controls, indicating that the composites do not instigate the desired local mineralization that we hypothesize to be important for interfacial design. However, since chondrocytes cultured on porous bioactive glass [9] did show a markedly higher alkaline phosphatase activity compared with collagen controls, one can expect that the tendency to develop the proper phenotype for calcification may be engineered into the composites by incorporating more glass particles. Preliminary cell culture results for polyurethane-based composites were similar to those shown here. Data are not yet available for polylactide-based materials.

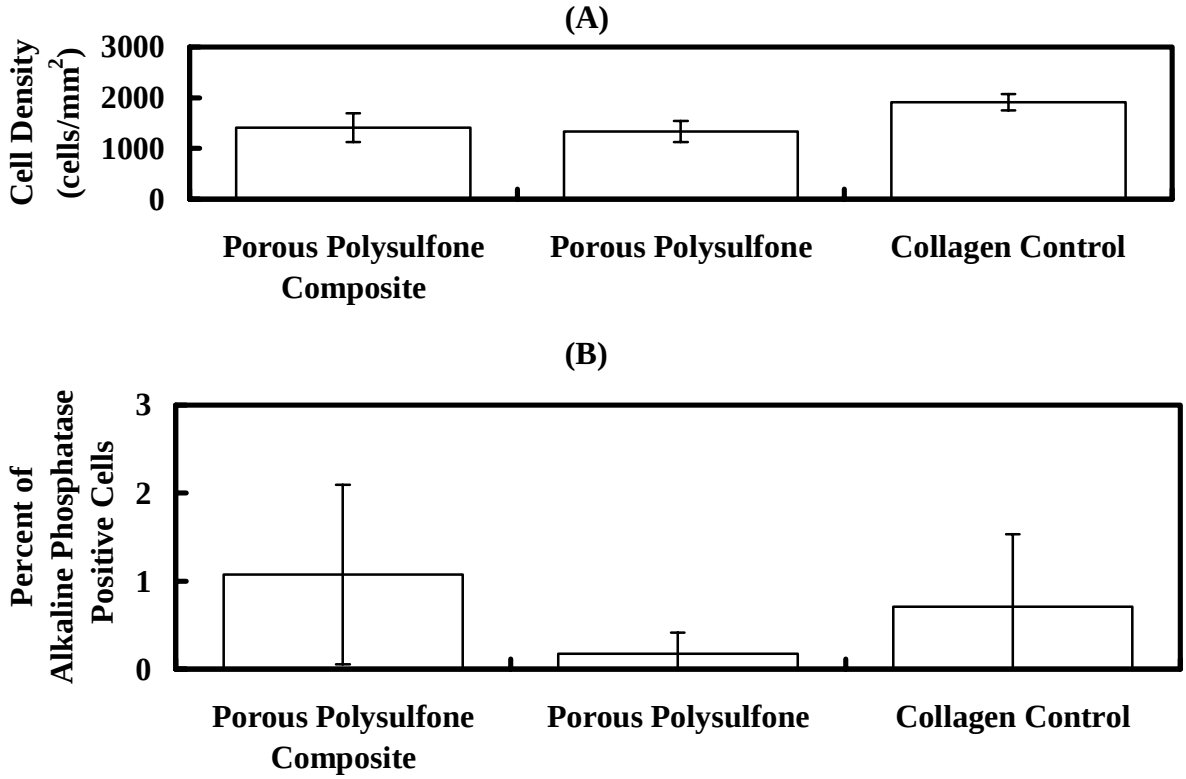

Figure 3. Cell density (A) and percent of alkaline phosphatase-positive cells (B) on porous polysulfone alone, porous polysulfone/bioactive glass composites (9 vol% glass) and Type I collagen control after culturing with rabbit chondrocytes for 2 weeks.

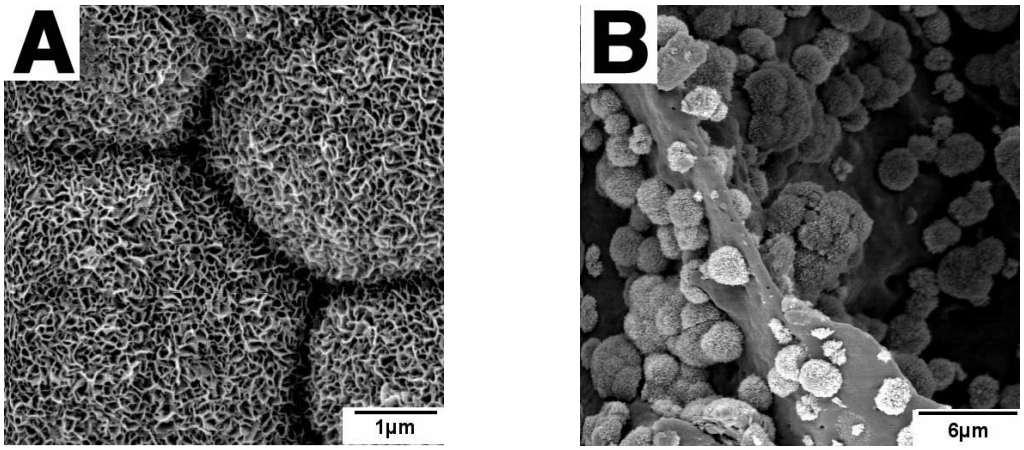

Figure 4. SEM images of the apatite formed on (A) and inside (B) the porous polyurethane/bioactive glass composites with 9 vol% glass.

The microstructure of porous polyurethane/bioactive glass composites after soaking in SBF for 2 weeks is shown in Figure 4. A new material with a fine flake-like structure appeared on the surfaces and the inside of composites. X-ray diffraction and FTIR results (not shown)

showed that the new material is crystalline hydroxycarbonate apatite (HCA). Similarly, HCA also developed inside and on porous polysulfone and polylactide composites after 2 weeks of soaking in SBF. Previous research has correlated the ability of biomaterials to develop HCA upon soaking in SBF to bone bonding *in vivo* [10]. Therefore, the *in vitro* formation of HCA in porous polymer/bioactive glass composites after soaking in SBF demonstrates their potential bone bonding ability.

CONCLUSIONS

Porous composites consisting of polymer (polysulfone, polyurethane or polylactide) and bioactive glass particles were produced by a phase separation technique. The composites have asymmetric structures with dense top layers and porous structures beneath. The dense top layer could be removed to make a structure with exposed large pores (20-150 μm). Culture studies revealed that polysulfone-based composite surfaces were suitable for attachment, spread and proliferation of chondrocytes. HCA growth inside and on the composites after soaking in SBF suggests the potential bone-bonding ability of the composites. These porous composites have potential applications as interface materials between soft and hard tissues, such as the artificial cartilage/bone interface.

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